

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

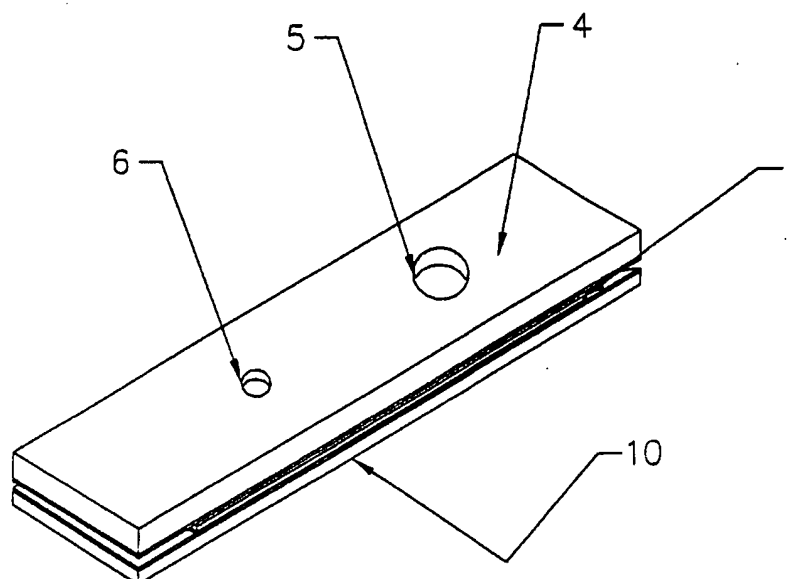
(51) International Patent Classification <sup>6</sup> : <b>G01N 33/487</b>	<b>A1</b>	(11) International Publication Number: <b>WO 99/17115</b>
		(43) International Publication Date: 8 April 1999 (08.04.99)

(21) International Application Number: PCT/US98/20575

(22) International Filing Date: 30 September 1998 (30.09.98)

(30) Priority Data:  
60/060,488 30 September 1997 (30.09.97) US(71) Applicant: MERCURY DIAGNOSTICS, INC. [US/US]; 4742  
Scotts Valley Drive, Scotts Valley, CA 95066 (US).(72) Inventors: DOUGLAS, Joel, S.; 25285 La Loma Drive, Los  
Altos Hills, CA 94022 (US). ROE, Jeffrey, N.; 3212  
Veracruz Drive, San Ramon, CA 94583 (US). PRIEST,  
John, H.; 2921 Panaview Boulevard, Everett, WA 98203  
(US). HASKER, David, A.; 6122 El Toro Court, San Jose,  
CA 95123 (US).(74) Agent: DILLAHUNTY, T., Gene; Burns, Doane, Swecker &  
Mathis, L.L.P., P.O. Box 1404, Alexandria, VA 22313-1404  
(US).(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR,  
BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD,  
GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR,  
KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN,  
MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK,  
SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO  
patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian  
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European  
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR,  
IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF,  
CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).**Published***With international search report.**Before the expiration of the time limit for amending the  
claims and to be republished in the event of the receipt of  
amendments.*

(54) Title: MEMBRANE BASED ELECTROCHEMICAL TEST DEVICE AND RELATED METHODS



## (57) Abstract

An electrochemical test device is provided for determining the presence or concentration of an analyte in an aqueous fluid sample. The electrochemical test device includes a working electrode and a counter electrode made of an amorphous semiconductor material. The working electrode is overlaid with a reagent capable of reacting with an analyte to produce a measurable change in potential which can be correlated to the concentration of the analyte in the fluid sample. The test device optionally contains a reference electrode made of an amorphous semiconductor material having a reference material on the reference electrode.

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

## MEMBRANE BASED ELECTROCHEMICAL TEST DEVICE AND RELATED METHODS

### BACKGROUND OF THE INVENTION

#### 5 Field of the Invention

The present invention relates to an electrochemical test device suitable for determining the presence or concentration of chemical and biochemical components (analytes) in aqueous fluid samples and body fluids such as whole blood or interstitial fluid. Additionally, this invention relates to a method of using such test devices for  
10 determining the presence or concentration of an analyte and to processes for preparing such a test devices.

#### State of the Art

Medical studies have demonstrated that the incidence of serious complications  
15 resulting from diabetes, such as vision loss and kidney malfunction, can be significantly reduced by careful control of blood glucose levels. As a result, millions of diabetics use glucose testing devices daily to monitor their blood glucose concentrations. Additionally, a wide variety of other blood testing devices are used to determine the presence or concentration of other analytes, such as alcohol or cholesterol, in aqueous samples, such  
20 as blood.

Such blood testing devices typically employ either a dry chemistry reagent system or an electrochemical method to test for the analyte in the fluid sample. In recent years, electrochemical testing systems have become increasingly popular due to their small size and ease of use. Such electrochemical testing systems typically use electrochemistry to  
25 create an electrical signal which correlates to the concentration of the analyte in the aqueous sample.

Numerous electrochemical testing systems and related methods are known in the art. For example, European Patent Publication No. 0 255 291 B1, to Birch et al., describes methods and an apparatus for making electrochemical measurements, in  
30 particular but not exclusively for the purpose of carrying out microchemical testing on small liquid samples of biological, e.g. of clinical, origin.

European Patent Publication No. 0 351 891 B1, to Hill et al., teaches a method of making an electrochemical sensor by printing. The sensor is used to detect, measure or monitor a given dissolved substrate in a mixture of dissolved substrates, most specifically glucose in body fluid.

5 U.S. Patent No. 5,391,250, to Cheney II et al., teaches a method of fabricating thin film electrochemical sensors for use in measuring subcutaneous or transdermal glucose. Fabrication of the sensors comprises placing a thin film base layer of insulating material onto a rigid substrate. Conductor elements for the sensors are formed on the base layer using contact mask photolithography and a thin film cover layer.

10 U.S. Patent No. 5,437,999, to Diebold et al., teaches a method of fabricating thin film electrochemical devices which are suitable for biological applications using photolithography to define the electrode areas. The disclosures of each of the above patent specifications are incorporated herein by reference in their entirety.

An excellent reference on materials and process for fabricating electronic  
15 components is Charles A. Harper, Handbook of Materials and Processes for Electronics, 1984, Library of Congress card number 76-95803. It provides detail process information on thick film, thin film and photo resist processes.

Existing electrochemical testing systems, however, have certain limitations from the perspective of the end user or the manufacturer. For example, some electrochemical  
20 testing systems are difficult or costly to manufacture. As a result, such devices are too expensive to be used on a daily basis by, for example, diabetics. Other electrochemical testing systems are not sufficiently accurate to detect certain analytes at very low concentrations or to give reliable measurements of the analyte's concentration. Additionally, many electrochemical devices are too large to be easily carried by those  
25 needing to test their blood on a regular basis throughout the day. Thus, a need exists for improved electrochemical test devices.

### SUMMARY OF THE INVENTION

The present invention utilizes amorphous semiconductor applied with thin film manufacturing techniques and membranes which have a skin on each planar surface to provide an electrochemical test device suitable for determining the presence or  
5 concentration of analytes in aqueous fluid samples. By using amorphous semiconductor materials applied with film manufacturing techniques and dual skin membranes, uniform electrochemical test devices having well-defined reproducible electrode areas can be manufactured economically.

In particular, the test devices of this invention have very uniform surface areas  
10 which reduce the variability of the electrochemical test. In this regard, it has been found that the surface area of the electrodes and the accuracy of applying the reagent are critical to producing an accurate test. If the surface area is not consistent from test to test then each of the test devices must be individually calibrated to insure accurate readings. The test devices of the present invention permit highly accurate electrochemical analyte  
15 measurements to be performed on very small aqueous fluid samples without the need for individual calibration of each test device. The present inventions provide for the accurate reproduction of the test devices by using controlled deposition methods, such as sputtering or vapor deposition and smooth skin membranes to form the electrodes with consistent size and surface morphology from device to device in continuous production.  
20 These devices can also be readily manufactured due to the lower cost and the flexible nature of the amorphous semiconductor materials which facilitates production by continuous roll processing versus the step and repeat printing methods currently employed. The ability to use continuous processing to fabricate the device, such as continuous processes utilizing continuous roll coating, continuous roll sputtering,  
25 continuous systems utilizing contact masks, results in high volume manufacturing capability and substantial cost reductions over the step and repeat processes. Additionally, the amorphous nature of the conductors electrodes and constructed and used according to this invention eliminates problems found in prior test devices which utilize conventional conductor and semiconductor materials, which are crystalline in  
30 nature or are noble metals and, as a result, require flat and rigid substrates to prevent

cracking during manufacture, distribution or use. The membrane in the present invention is the carrier for the indicating reagent and forms the surface for the electrode formation. The membrane comprises the matrix in which the reagent is carried or impregnated and comprises the two exterior skin surfaces on which the electrodes are placed. The skin  
5 surfaces can be smooth skin suitable for carrying the electrodes and can be porous to pass the aqueous fluid samples or can have pores sized to screen or block selected components from aqueous fluid sample, such as red blood cells in a blood sample.

Dry electrochemical test devices fall into two primary configurations. The first configuration utilizes two electrodes, i.e., a working electrode and a counter electrode.  
10 The second configuration utilizes three electrodes, i.e., a working electrode, a counter electrode and a reference electrode. The use of the reference electrode and a reference material provides a fixed reference for the test. The test devices of the present invention are well suited for a two electrode system however, a contact mask could be employed during sputtering to create a surface with two electrodes.

15 Accordingly, in one of its aspects, the present invention provides an electrochemical test device for determining the presence or concentration of an analyte in an aqueous fluid sample, said electrochemical test device comprising:

- (a) a non-conductive surface;
- (b) a working electrode comprising an amorphous semiconductor material  
20 affixed to the non-conductive surface of a double skin membrane, said working electrode having an first electrode area, a first lead and a first contact pad;
- (c) a counter electrode comprising an amorphous semiconductor material affixed to the opposite non-conductive surface of a double skin membrane, said counter electrode having an second electrode area, a second lead and a second contact pad; and  
25 (d) a reagent capable of reacting with the analyte to produce a measurable change in potential which can be correlated to the concentration of the analyte in the fluid sample, said reagent being imbibed into the membrane matrix between the two electrode surfaces.

In another embodiment of this invention, the test device further comprises a  
30 reference electrode comprising an amorphous semiconductor material affixed to the non-

conductive surface, said reference electrode having an electrode area, a lead, and a contact pad, and wherein at least a portion of the electrode area is overlaid with a reference material. Preferably, the reference material is a silver/silver chloride layer, a mercury/mercury chloride layer or a platinum/hydrogen material. This electrode could be  
5 either the counter electrode or an independent third electrode.

In a preferred embodiment of this invention, the test device further comprises a blood separating membrane with two skin surfaces. The blood separating membrane separates whole blood samples into highly colored and relatively clear fluid portions before analysis. The blood separating membrane effectively blocks or filters red blood  
10 cells and allows essentially clear fluid to pass to the test reagent imbibed in the membrane matrix. As a result, the analyte is measured in the clear fluid portion of the sample contacting the electrodes thereby substantially eliminating the red blood cells from the reaction and minimizing interference from the cells present in blood. This embodiment has the additional benefit of keeping the test site from drying out and thereby improves  
15 the performance of test devices designed for small sample sizes, such as in the 1 to 5 microliter range.

Preferably, the membrane is selected from polysulphone, polyethersulphone, or nylon and is cased with a tight pore skin on each side and a relatively isotropic matrix between each skin surface

20 The skin pore size is relatively tight approximately 0.1 to 0.5 microns in size and the isotropic matrix being 0.5 microns or greater in pore size. The tight pore size provides a uniform surface morphology on which the amorphous semiconductor electrodes are formed according to this invention. Better surface morphology of the membrane results in a more consistent surface for the amorphous semiconductor electrodes. This provides  
25 improved accuracy of test results and consistency of performance.

Preferably, the amorphous semiconductor material used in this invention is amorphous silicon oxide. More preferably, the amorphous silicon oxide is doped with lithium, potassium, or a similar conducting ion to increased conductivity. Doping with lithium is particularly preferred. However, amorphous carbon, gold, silver or other  
30 conductor materials which do not interfere with the electrochemistry of the reagent

system are also suitable. The amorphous semiconductor material can be applied by using various techniques including sputtering, evaporation, vapor phase deposition or other thin film deposition technique to form a conductive layer on the membrane surface. The surface texture of the amorphous semiconductor material is preferably less than 13 micro inches or 0.33 microns. However, rougher textures can be used depending on the accuracy of the desired test device.

The reagent employed in the electrochemical test device is typically selected based on the analyte to be tested and the desired detection limits. The reagent preferably comprises an enzyme and a redox mediator. When the analyte to be detected or measured is glucose, the enzyme is preferably glucose oxidase and the redox mediator is potassium ferrocyanide.

The electrochemical test device of the present invention is used to determine the presence or concentration of an analyte in an aqueous fluid sample. Accordingly, in one of its method aspects, the present invention provides a method for determining the presence or concentration of an analyte in an aqueous fluid sample, said method comprising:

- (a) providing an electrochemical test device comprising: (i) double skinned membrane; (ii) a working electrode comprising an amorphous semiconductor material affixed to the membrane surface, said working electrode having a first electrode area, a first lead and a first contact pad area; (iii) a counter electrode comprising an amorphous semiconductor material affixed to the non-conductive surface, said counter electrode having a second electrode area, a second lead, and a second contact pad; and (iv) a reagent capable of reacting with the analyte to produce a measurable change in potential which can be correlated to the concentration of the analyte in the fluid sample, said reagent being imbibed into the membrane matrix between the two electrode surfaces;
- (b) inserting the electrochemical test device into a meter device;
- (c) applying a sample of an aqueous fluid to the membrane area of the working electrode;
- (d) reading the meter device to determine the presence or concentration of the analyte in the fluid sample.



In another embodiment, the test device employed in this method further comprises a reference electrode comprising an amorphous semiconductor material affixed to the counter electrode membrane surface, said reference electrode having a third electrode area, a third lead, and a third contact pad, and wherein at least a portion of the third  
5 electrode area is overlaid with a reference material.

Preferably, the reference material is a silver/silver chloride layer, a mercury/mercury chloride layer or a platinum/hydrogen material. Silver/silver chloride is a particularly preferred reference material. The separation of the counter electrode conductive path and reference electrode is accomplished by using a mask to create the  
10 different geometries on the same surface.

Preferably, the membrane is selected from polysulphone, polyethersulphone, or nylon and is cased with a tight pore skin on each side and a relatively isotropic matrix between each skin surface

The skin pore size is relatively tight approximately 0.1 to 0.5 microns in size and  
15 the isotropic matrix being 0.5 microns or greater in pore size. The tight pore size provides a uniform surface morphology on which the amorphous semiconductor electrodes are formed according to this invention. Better surface morphology of the membrane results in a more consistent surface for the amorphous semiconductor electrodes. This provides improved accuracy of test results and consistency of performance.

20 As discussed above, the present invention utilizes amorphous semiconductor materials and thin manufacturing techniques to provide electrochemical test devices. Thin film technologies can be used to create the amorphous semiconductor material conductive layers and electrodes according to this invention. Accordingly, in one of its process aspects, the present invention provides a process for preparing an electrochemical  
25 test device suitable for determining the presence or concentration of an analyte in an aqueous fluid sample, said process comprising the steps of:

- (a) providing a skinned membrane having a first and an opposite second surface;
- (b) depositing an amorphous semiconductor material on said first surface to  
30 form a conductive layer;

(c) depositing an amorphous semiconductor material on said opposite second surface to form a conductive layer

(d) applying a reagent to the membrane which is imbibed into the membrane matrix between the two surfaces, said reagent being capable of reacting with an analyte in  
5 an aqueous fluid sample to produce a measurable change in potential which can be correlated to the concentration of the analyte in the fluid sample.

In another embodiment, step (c) of this process further comprises forming a reference electrode comprising a third electrode having a third electrode area, a third lead and a third contact pad. This is accomplished by using a mask to form the two distinct  
10 electrodes and in a step (e) forming a silver chloride surface.

In a preferred embodiment, step (a) above comprises the steps of:

(f) providing a double skinned membrane with the correct pore size distribution; and

In another preferred embodiment, step (d) above comprises the steps of:

(h) positioning a mask on the opposing membrane surface and sputtering an  
15 amorphous conductive surface to the membrane surface to form independent electrodes;

(i) said second exposed conductive surface area comprising (i) a counter electrode comprising a second electrode having a second electrode area, a second lead and a second contact pad, and optionally (ii) a reference electrode comprising a third  
20 electrode having a third electrode area, a third lead and a third contact pad.

In further preferred embodiment, step (d) above further comprises the steps of:

(o) positioning a second mask on the opposing surface so that the opposing electrode area is masked and the third reference electrode area is exposed;

(p) applying a reference material the third electrode area;

25 (q) removing the mask.

Preferably, the process employed to prepare the test devices of this invention is a continuous process. The ability to use continuous processing to fabricate the test devices, such as a continuous process utilizing continuous roll coating, continuous roll sputtering, continuous sputtering systems utilizing contact masks, results in high volume

manufacturing capability and substantial cost reductions over the step and repeat printing processes.

### BRIEF DESCRIPTION OF DRAWINGS

5           Figure 1 illustrates a plan view of the membrane showing the top side and amorphous semiconductor coating.

          Figure 2 illustrates a side view of the membrane showing the skin and amorphous semiconductor coatings.

          Figure 3 illustrates a plan view of the membrane showing the bottom side and  
10   amorphous semiconductor coating.

          Figure 4 illustrates an isometric view of the non conductive top layer.

          Figure 5 illustrates an isometric view of the double skin membrane with conductive layers.

          Figure 6 illustrates an isometric view of the non conductive bottom layer.

15           Figure 7 illustrates an isometric view of the assembled device.

          Figure 8 illustrates finished reference and opposing electrode.

          Figure 9 illustrates an isometric view of membrane position in a sputter frame.

### DETAILED DESCRIPTION OF THE INVENTION

20           The present invention utilizes amorphous semiconductor materials and double skin membranes to provide an electrochemical test device suitable for determining the presence or concentration of analytes in aqueous fluid samples. By using amorphous semiconductors and the double skinned membranes, uniform electrochemical test devices having well-defined reproducible electrode areas can be manufactured economically.

25           These areas have very uniform surface morphology which reduces the variability of the electrochemical test. The surface morphology is directly related to the surface area of the electrodes. The amount and concentration of applied reagents are also critical to producing an accurate test. If the surface area is not consistent from device to device then the individual devices manufactured have to be individually calibrated to insure accurate

30           readings in a meter. The test devices of the present invention permit highly accurate

electrochemical analyte measurements to be performed on very small aqueous fluid samples due to the accurate reproduction of the test devices using controlled deposition methods such as sputtering, vapor phase deposition, glow discharge methods or evaporation and the very uniform surface of the double skin membranes to form the geometries. These devices can also be readily manufactured due to the lower cost and flexible nature of the amorphous semiconductor materials which allow the use of volume production in continuous processing manufacturing methods.

Thin film technologies are preferred for forming the amorphous semiconductor material layer or coating, such as sputtering, vapor phase deposition, glow discharge methods or evaporation. Such processes are capable of producing coatings up to a thickness of a few microns and of applying the coatings uniformly over the entire surface. If desired, thick film technologies are capable of producing much thicker layers in the range of less than 0.005 inches. While both methods can produce uniform surfaces, thin film surface morphology is dependent on the surface roughness of the underlying substrate, whereas the thick film technologies are capable of changing the final surface morphology of the device due to their thicker structure.

The electrochemical test device of this invention employs at least two types of electrodes. The first type of electrode is referred to as the "working or indicating electrode" and the second type is referred to as the "counter or opposing electrode". These electrodes permit changes in potential between the electrodes to be accurately measured when the analyte is applied across the electrodes, and the reagent reactive with the analyte is bound to the membrane matrix between the electrodes formed on the skin. Optionally, the test device may also contain a reference electrode. The reference electrode allows accurate measurements of potential to be made with respect to a fixed reference point. Any suitable reference electrode may be used, include silver/silver chloride, mercury/mercury chloride and platinum/hydrogen reference electrodes. Preferably, the reference electrode is a silver/silver chloride reference electrode and the working electrode has a potential ranging from about +1 to -1 volts versus the reference electrode. Reference electrodes suitable for use in this invention are further described in

R. S. C. Cobbold, *Transducers for Biomedical Measurements: Principles and Applications*, pp. 343-349, John Wiley & Sons (New York).

The electrodes in the test device of this invention are manufactured using a double skinned membrane. Any suitable dielectric material may be used as the material for the membrane matrix as long as a tight pore skin can be formed on both sides of the  
5 membrane. For example, polysulphone, polyethersulphone, or nylon and is casted with a tight pore skin on each side and a relatively isotropic matrix between each skin surface. The skin pore size is relatively tight approximately 0.1 to 0.5 microns in size and the isotropic matrix being 0.5 microns or greater in pore size.. Such polymeric materials are  
10 well known in the art and are commercially available. The membranes employed in preferred embodiments of this invention is Supor 200 available from Pall Gelman Sciences

The electrodes are then prepared on the membrane using semiconductor manufacturing techniques. Typically, a conductive layer is first deposited on or applied  
15 to the membrane. The conductive layer comprises an amorphous semiconductor material, such as amorphous silicon oxide, amorphous carbon, gold or silver and the like. Amorphous metals can be formed by permitting a small amount of helium gas to escape in the sputtering chamber during sputtering. Such materials are well known in the art. For example, formation of suitable amorphous semiconductor films are described in U.S.  
20 Patent No. 4,226,898, to Ovshinsky, the disclosure of which is incorporated herein by reference in its entirety.

Preferably, the amorphous semiconductor material employed in this invention is doped with lithium, potassium, or a similar conducting ion to increased conductivity. U.S. Patent Nos. 3,983,076, and 4,178,415, to Ovshinsky, describe suitable methods for  
25 adding various compounds to amorphous semiconductor material to increase its conductivity. The disclosures of these patents are incorporated herein by reference in their entirety.

The amorphous semiconductor material is preferably deposited on the non-conducting membrane skin to form a conductive layer having a thickness of less than  
30 about 0.005 inches, more preferably a thickness ranging from about 1 to about 5 microns.

The amorphous semiconductor material may be applied to the membrane using any art recognized procedure to form a conductive layer, such as sputtering, vapor phase deposition, glow discharge deposition, evaporation and the like. Alternatively, other deposition techniques such as electroplating, thick film laminating, roll transfer and the like may be employed. Thinner films are best achieved by sputtering, evaporation, vapor phase deposition, glow discharge methods or other thin film deposition method whereas thicker films are best formed using thick film deposition techniques such as electroplating, thick film laminating, roll transfer and the like. Charles A. Harper, *Handbook of Materials and Processes for Electronics*, 1984, (Library of Congress card number 76-95803) discusses various methods of forming thin films in Chapter 11, describes various thick film process in Chapter.

Preferably, the amorphous semiconductor material is deposited using vapor phase deposition or sputtering techniques. In a particularly preferred embodiment, the amorphous semiconductor material is formed by co-deposition of the amorphous semiconductor material and the doping material such as by co-vacuum deposition or co-sputtering. Co-sputtering can be accomplished in a conventional DC or RF. sputtering system, such as available from ULVAC Vacuum Systems. In this process, a cathode or target is bonded to a standard aluminum backing plate and is made of the semiconductor material to be deposited. Also, pieces of the doping material are secured to the cathode or target. Substrates upon which the amorphous semiconductor material and the doping material are to be deposited are carried by a holder located from the target by a distance of about 3.5 cm for a 3 1/3" diameter cathode or passed continuously by the target.

The sputtering machine is first evacuated to a vacuum pressure somewhat less than about  $6 \times 10^{-6}$  torr to provide a background vacuum pressure. Argon is bled into the machine to provide an operating pressure of about  $5 \times 10^{-3}$  torr as determined by a reading on a Pirani vacuum gauge, giving an actual vacuum pressure of about  $7 \times 10^{-3}$  torr in the machine. The surface of the cathode or target and pieces of doping material are first cleaned by sputtering against the shutter of the machine adjacent to substrates for about 30 minutes. Thereafter, the shutter is opened and the semiconductor material of the target and the pieces of doping material on the target are co-sputtered onto the non-conductive

coating or substrate. The cathode or target and the holder for the substrates are both water cooled so that their temperature is low during the sputtering operation, as for example, approximately 50°C. The RF. power of the machine may have a frequency of about 13.56 MegaHertz, and about 1000 Volts of forward power, about 50-70 Watts  
5 being utilized for 3.5" diameter cathode or target.

The deposition rates depend upon the materials being sputtered, and the time of deposition is varied to obtain desired thicknesses of the deposit. The thicknesses of the simultaneously deposited amorphous semiconductor film having the doping material therein may vary from a few hundred Angstroms to about 5 microns (5 $\mu$ ), depending  
10 upon the uses to which the amorphous semiconductor film is to be put. The amorphous semiconductor material is deposited on the membrane.

The amount of doping material homogeneously incorporated in the amorphous semiconductor material is generally determined by the area of the pieces of the doping material applied to the semiconductor material of the cathode or target. Desired  
15 percentage of modifier material added to the amorphous semiconductor material may accordingly be conveniently controlled. By utilizing co-sputtering as generally here described, the doping material is substantially homogeneously incorporated in the semiconductor material to provide the beneficial features of this invention.

Preferably, the amorphous semiconductor material is applied continuously to the  
20 membrane using art recognized continuous processes. Such continuous processes are described, for example, in U.S. Patent No. 3,983,076, to Rockstad, and in U.S. Patent Nos. 3,271,591 and 4,178,415, to Ovshinsky, the disclosures of which are incorporated herein by reference in their entirety. The ability to use continuous processing to fabricate the test devices of this invention, such as a continuous process utilizing continuous roll  
25 coating, continuous roll sputtering, results in high volume manufacturing capability and substantial cost reductions over the step and repeat processes currently used. Suitable machines for such continuous processes are made by Ulvac Vacuum Systems of Japan.

Optionally, if a reference electrode is used, the third electrode, if present, is constructed by using a contact mask which isolates the two electrodes formed on the  
30 bottom skin. The contact mask permits only application of sputtered material in the open

areas and does not permit the coating on the covered areas. The reference electrode is then converted into a reference electrode by applying a suitable reference material.

Suitable reference materials include silver/silver chloride, a mercury/mercury chloride and platinum/hydrogen materials. Such materials can be applied to the third electrode  
5 area of the reference electrode by any thin film deposition method described above.

A particularly preferred reference material is silver/silver chloride. In order to obtain consistent results from device to device, the silver/silver chloride layer must be applied such that the silver/silver chloride layer covers essentially the same surface area in each device manufactured. Preferably, this is accomplished using contact mask as  
10 described above to expose a precise area of the electrode after developing. A thin layer of silver is then deposited on the exposed area, preferably by sputtering or other consistent film application method. The silver layer is preferably less than about 0.001 inches in thickness, more preferably about 1 micron to about 5 microns in thickness. Alternatively, a silver layer can be applied to the electrode area of the reference electrode using pad  
15 printing, inkjet methods, transfer roll printing or similar techniques. The silver layer is then contacted with  $\text{FeCl}_3$  solution to convert the silver surface to silver chloride thereby forming a silver/silver chloride layer

The electrochemical test device is then completed by applying an appropriate reagent to the membrane. The reagent is imbibed into the membrane matrix under the  
20 sample application area. Suitable reagents for determining the presence or concentration of various analytes are well known in the art and are described in further detail herein below.

#### Preferred Process for Preparing the Electrodes

25 As described above, the electrodes for the electrochemical test device of this invention are prepared using amorphous semiconductor materials and double sided skinned membrane. Preferably, such electrodes are formed in a continuous manner. A preferred process for the preparation of electrodes suitable for use in this invention is illustrated in Figures 1-9.



As illustrated in Figures 1, 2 and 3, the membrane 1 which has skins 12 and 13 is coated with amorphous semiconductor material creating surfaces 2 and 4 which are the electrodes have a thickness of less than about 0.005 inches and preferable in the range of 1 to 5 microns.

5           Figure 4 shows an isometric view of the top non conductive layer 4 which has been coated with adhesive 7. Non conductive layer 4 has holes 5 and 6 punched in the layer. Hole 5 is the sample application hole and permits the application of the indicating reagent during manufacture. Hole 6 is permits the measuring device to contact the conductive surface of the membrane.

10           Figure 5 shows an isometric view of the membrane with conductive layers 2 and 4 and reference coating 14 (not shown) applied to the opposite side of the membrane.

            Figure 6 shows an isometric view of the bottom non conductive layer 10 which has been coated with adhesive 8. Non conductive layer 10 has holes 9 and 11 punched in the layer. Hole 9 is the sample vent hole and permits the venting of the air in the  
15           membrane during application of the indicating reagent when the strip is manufactured. Hole 11 is permits the measuring device to contact the conductive surface of the membrane.

            Figure 7 is an isometric view of the finished device showing the membrane 1 sandwiched between the top and bottom non conductive layers 4 & 10 with top vent hole  
20           5 and top contact hole 6.

            As shown in cross section Figure 8 is a bottom view of a membrane showing opposing electrode 3 and reference electrode 15 with reference coating 14. The pattern is formed by sputtering on the skin and using a contact mask to pattern the electrodes.

            As illustrated in Figure 9, the membrane 1 can be loaded into a frame 20 to permit  
25           discrete manufacturing.

#### Reagents

            Various types of analytical or electrochemical sensor reagents may be applied to the electrodes. To create a functional electrochemical test device, a reagent chemistry  
30           must be selected based on the analyte to be tested and the desired detection limits.

Preferably, the reagent is deposited on the membrane such that a uniform amount is applied from sensor to sensor. The reagent may be applied using any conventional procedure, such as nozzle coating through the hole 5 in layer 4 using IVEK pumps or any other drop on demand system capable of delivering consistent and uniform volume of reagent.

The reagent is applied through the working electrode, but may in some cases also be applied through the other electrodes. After the reagent has been imbibed into the membrane, it is typically dried. Subsequently, when the test device is used, the test sample of aqueous fluid, such as blood, rehydrates the reagent and a potential is applied to the electrodes from which a current measurement may be taken by a meter. The membrane matrix acts as reagent carrier and electrode spacer. This permits the device to have a reduced manufacturing cost due to the excellent uniformity of the membrane thickness and isotropic nature of the inner matrix of the membrane.

An example reagent formulation suitable for use in the present invention is described below. This reagent may be used to determine the presence or concentration of glucose in an aqueous fluid sample. Preferably, this reagent formulation is used with an electrochemical sensor having an opposing electrode 3, working electrode 2 and reference electrode 15.

Reagent Formulation

Material	Amount/Concentration
2-(N-morpholino)ethanesulfonic acid (MES buffer)	100 millimolar (mM)
Triton X-100	0.08% wt/wt
Polyvinyl alcohol (PVA) mol. wt. 10K 88% hydrolyzed	1.00% wt/wt
Imidazole osmium mediator, reduced, as defined in U.S. Patent No. 5,437,999	6.2 mM
Glucose Oxidase	6000 units/mL

The above reagent formulation may be prepared using the following procedures:

(a) 1.952 grams of MES buffer is added to 85 mL of nanograde water. The mixture is stirred until the components dissolve. The pH of the solution is adjusted to 5.5 with NaOH. The volume of the solution is then brought to 100 mL of final buffer solution.

(b) 0.08 grams of Triton X-100 and 1 gram of PVA is added to a beaker capable of holding all the components (e.g., a 200 mL beaker). The buffer solution is added to bring the total solution weight to 100 grams. The mixture is heated to boiling and stirred to dissolve the PVA.

(c) 4.0 mg of the reduced osmium mediator is added to 1 mL of the solution from step (b) above. The mixture is stirred to dissolve the mediator.

(d) The mixture is left to cool to room temperature.

(e) 6000 units of glucose oxidase are added and the mixture is mixed until the enzyme is dissolved.

The above reagent formulation may be used to determine the presence or concentration of glucose in an aqueous fluid sample. As will be apparent to those skilled in the art, other reagent formulations may be employed to assay different analytes. Such reagent formulations are well known in the art. Typically, such reagent formulations are designed to react specifically with the desired analyte to form a measurable electrochemical signal.

Without being limited to theory, it is believed that in the example reagent formulation described above, glucose is anaerobically oxidized or reduced with the involvement of the enzyme and the redox mediator. Such a system is sometimes referred to as an amperometric biosensor. Amperometry refers to a current measurement at constant applied voltage on the working electrode. In such a system, the current flowing is limited by mass transport. Therefore, the current is proportional to the bulk glucose concentration. The analyte, enzyme and mediator participate in a reaction where the mediator is either reduced (receives at least one electron) or oxidized (donates at least one electron). The glucose reaction ends when glucose oxidase is oxidized and the mediator is reduced. The mediator is then oxidized at the surface of the working electrode by the

applied potential difference. Changes in the system amperage result from changes in the ratio of oxidized/reduced form of the redox mediator. The amperage change directly correlate to the detection or measurement of glucose in the test sample.

Various enzymes may be used in the reagent formulations employed in this invention. The particular enzyme employed will vary depending on the analyte to be detected or measured. Preferred enzymes include glucose oxidase, glucose dehydrogenase, cholesterol esterase and alcohol oxidase. The amount of enzyme employed will generally range from about 0.5 to about 3.0 million units of enzyme per liter of reagent formulation.

The reagent formulation will also typically contain a redox mediator. The redox mediator will generally be chosen to be compatible with the enzyme employed and combinations of redox mediators and enzymes are well known in the art. Suitable redox mediators include, by way of example, potassium ferricyanide and ferrocene derivatives, such as 1,1'-dimethyl ferrocene. The amount of redox mediator employed in the reagent formulation will typically range from about 0.15 M to about 0.7 M. Additional mediators suitable for use in this invention include methylene blue, *p*-benzoquinone, thionine, 2,6-dichloroindophenol, gallocyanine, indophenol, polyviologen, osmium bis(2,2'-bipyridine) dihydrochloride, and riboflavin-5'-phosphate ester. Optionally, these mediators can be chemically bound or entrapped in a matrix, such as a polymer, using procedures well known in the art.

Examples of enzyme/mediator combinations suitable for use in this invention include, but are not limited to, the following:

<u>Analyte</u>	<u>Enzyme</u>	<u>Mediator</u>
glucose	glucose dehydrogenase	ferricyanide
glucose	glucose oxidase	tetracyanoquinodimethane
cholesterol	cholesterol esterase	ferricyanide
alcohol	alcohol oxidase	phenylenediamine

A preferred reagent chemistry uses potassium ferricyanide as a mediator.

In addition to an enzyme and a redox mediator, the reagent layer on the electrode preferably further comprises a buffer, a stabilizer, a dispersant, a thickener or a surfactant. These materials are typically employed in amounts which optimize the reaction of the reagents with the analyte. The concentration ranges for these components referred to  
5 below are for the reagent formulation before it has dried on the electrode surface.

A buffer is preferably employed in the reagent formulation to provide a satisfactory pH for enzyme function. The buffer used must have a higher oxidation potential than the reduced form of the redox mediator. A preferred buffer for use in this invention is a phosphate buffer having a concentration ranging from about 0.1 M to about  
10 0.5 M. Other suitable buffers include BES, BICINE, CAPS, EPPS, HEPES, MES, MOPS, PIPES, TAPS, TES and TRICINE buffers (collectively known as 'GOOD' buffers), citrate, TRIS buffer, and the like. The 'GOOD' and TRIS buffers are commercially available from Sigma-Aldrich, Inc. (St. Louis, MO, USA).

A stabilizer may also be employed in the reagent formulation to stabilize the  
15 enzyme. When the enzyme used is glucose oxidase, a preferred stabilizer is potassium glutamate at a concentration ranging from about 0.01 to 4.0 % weight. Other suitable stabilizers include succinate, aspartate, blue dextran and the like.

Additionally, dispersants may be used in the reagent formulation to enhance the dispersion of the redox mediator and to inhibit its recrystallisation. Suitable dispersants  
20 include microcrystalline cellulose, dextran, chitin and the like. Typically, the dispersant is used in the reagent formulation in an amount ranging from about 1.0 to about 4.5 % weight. Preferred dispersants include, but are not limited to, AVICEL RC-591 (a microcrystalline cellulose available from FMC Corp.) and NATROSOL-250 M (a microcrystalline hydroxyethylcellulose available from Aqualon).

25 A thickener may also be employed in the reagent formulation to hold the reagent to the electrode surface. Suitable thickeners include water soluble polymers, such as polyvinylpyrrolidone.

Additionally, a surfactant may be added to the reagent formulation to facilitate rapid and total wetting of the electrode surface. Preferably, the reagent formulation

contains a nonionic surfactant in an amount ranging from about 0.01 to 0.3 % by weight. A preferred surfactant is TRITON X-100, available from Sigma-Aldrich, Inc.

#### Use of the Electrochemical Test Device

5           To illustrate the use of an electrochemical test device of this invention, the following glucose assay is described. It will be understood, however, that by selecting the proper reagent, other analytes may be determined using these procedures.

          The electrodes of the electrochemical test device are prepared as described above and the membrane is coated with 1.0  $\mu$ L of the above-described reagent formulation and  
10       dried.

          The electrochemical test device is then inserted in a meter before the test sequence is initiated. Any suitable meter device which has contacts that interface with the test device contacts may be employed. Such metering devices are well known in the art. The meter will generally contain a measuring circuit and be adapted to apply an algorithm to  
15       the current measurement whereby the analyte level is provided and visually displayed. Examples of suitable power sources and meters may be found, for example, in U.S. Patent Nos. 4,963,814, 4,999,632, and 4,999,582 to Parks et al., U.S. Patent No. 5,243,516 to White et al., and European Patent Application No. 89116797.5, to Hill et al. The disclosures of these patents are incorporated by herein by reference in their entirety.

20           A small sample of blood or other aqueous fluid is then applied to the test device. The current is measured about 10 to about 30 seconds after applying the sample. The current is read by the meter between the working and counter electrode and, optionally, is compared to the reference electrode, if it is present. The meter then applies the algorithm to the current measurement and converts the measurement to an analyte concentration.  
25       This analyte level is visually displayed on the meter.

          From the foregoing description, various modifications and changes in the electrochemical test devices, processes and methods of this invention will occur to those skilled in the art. All such modifications coming within the scope of the appended claims are intended to be included therein.

Additional materials and methods useful in this invention are disclosed in  
copending PCT International Publication No. WO 97/48979.

Claims:

1. An electrochemical test device for determining the presence or concentration of an analyte in an aqueous fluid sample, said electrochemical test device comprising:
  - 5 (a) a non-conductive membrane having a first skin and a second skin and a matrix therebetween;
  - (b) a working electrode comprising an amorphous semiconductor material affixed to the non-conductive membrane first skin, said working electrode having an first electrode area, a first lead and a first contact pad;
  - 10 (c) an opposing electrode comprising an amorphous semiconductor material affixed to the non-conductive membrane second skin, said opposing electrode having an second electrode area, a second lead and a second contact pad; and
  - (d) a reagent capable of reacting with the analyte to produce a measurable change in potential which can be correlated to the concentration of the analyte in the fluid  
15 sample, said reagent imbibed in the membrane matrix between the working electrode and opposing electrode.
2. The electrochemical test device of Claim 1 wherein said device further comprises a reference electrode comprising an amorphous semiconductor material affixed  
20 to the non-conductive membrane second skin, said reference electrode having a third electrode area, a third lead, and a third contact pad, and wherein at least a portion of the third electrode area is overlaid with a reference material.
3. The electrochemical test device of Claim 2 wherein said reference material  
25 is silver/silver chloride.
4. The electrochemical test device of Claim 1 wherein the non conductive membrane material is selected from polysulphone, polyethersulphone, or nylon and is cased with a tight pore skin on each side and a relatively isotropic matrix between the  
30 skin surfaces.



5. The electrochemical test device of Claim 1 wherein the amorphous semiconductor material is amorphous silicon oxide.

6. The electrochemical test device of Claim 1 wherein the reagent comprises  
5 a glucose oxidase and a redox mediator.

7. A method for determining the presence or concentration of an analyte in an aqueous fluid sample, said method comprising:

(a) providing an electrochemical test device comprising: (i) a non-conductive  
10 double skinned membrane; (ii) a working electrode comprising an amorphous semiconductor material affixed to the non-conductive membranes first skin surface, said working electrode having an first electrode area, a first lead and a first contact pad; (iii) a counter electrode comprising an amorphous semiconductor material affixed to the non-conductive membranes second skin surface, said counter electrode having a second  
15 electrode area, a second lead, and a second contact pad; and (iv) a reagent capable of reacting with the analyte to produce a measurable change in potential which can be correlated to the presence or concentration of the analyte in the fluid sample, said reagent imbibed into the membrane between the working and opposing;

(b) inserting the electrochemical test device into a meter device;

20 (c) applying a sample of an aqueous fluid to the first electrode area of the working electrode;

(d) reading the meter device to determine the presence or concentration of the analyte in the fluid sample.

25 8. The method of Claim 7 wherein the electrochemical test device further comprises a reference electrode comprising an amorphous semiconductor material affixed to the non-conductive membranes second surface, said reference electrode having a third electrode area, a third lead, and a third contact pad, and wherein at least a portion of the third electrode area is overlaid with a reference material.

30

9. The electrochemical test device of Claim 7 wherein the non conductive membrane material is selected from polysulphone, polyethersulphone, or nylon and is cased with a tight pore skin on each side and a relatively isotropic matrix between each skin surface .

5

10. The method of Claim 7 wherein the reagent comprises an enzyme and a redox mediator.

11. A process for preparing an electrochemical test device suitable for  
10 determining the presence or concentration of an analyte in an aqueous fluid sample, said process comprising the steps of:

- (a) providing a non-conductive double skinned membrane;
- (b) depositing an amorphous semiconductor material on said first skin surface to form a conductive layer which forms a working electrode comprising a first electrode  
15 having a first electrode area, a first lead and a first contact pad
- (c) depositing an amorphous semiconductor material on said second skin surface to form a conductive layer; which forms an opposing electrode comprising a second electrode having a second electrode area, a second lead and a second contact pad
- (d) applying a reagent to imbibe the membrane through the first electrode area  
20 of the working electrode, said reagent being capable of reacting with an analyte in an aqueous fluid sample to produce a measurable change in potential which can be correlated to the concentration of the analyte in the fluid sample.

12. The process of Claim 11 wherein step (c) further comprises forming a  
25 reference electrode on the second skin surface comprising a third electrode having a third electrode area, a third lead and a third contact pad.

13. The electrochemical test device of Claim 11 wherein the non conductive membrane material is selected from polysulphone, polyethersulphone, or nylon and is

cased with a tight pore skin on each side and a relatively isotropic matrix between each skin surface.

14. The process of Claim 11 wherein the reagent comprises a glucose oxidase  
5 and a redox mediator.

15. A process of Claim 12 wherein said process is continuous.

FIGURE 1

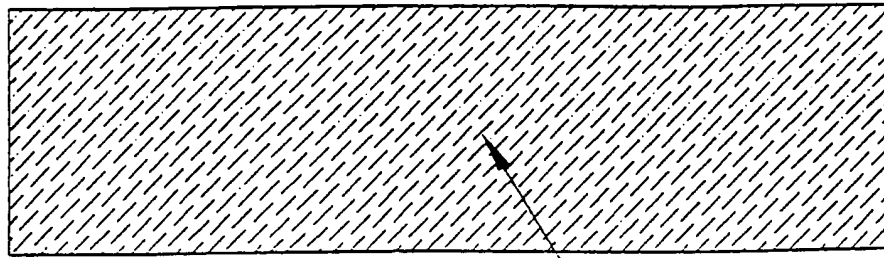


FIGURE 2

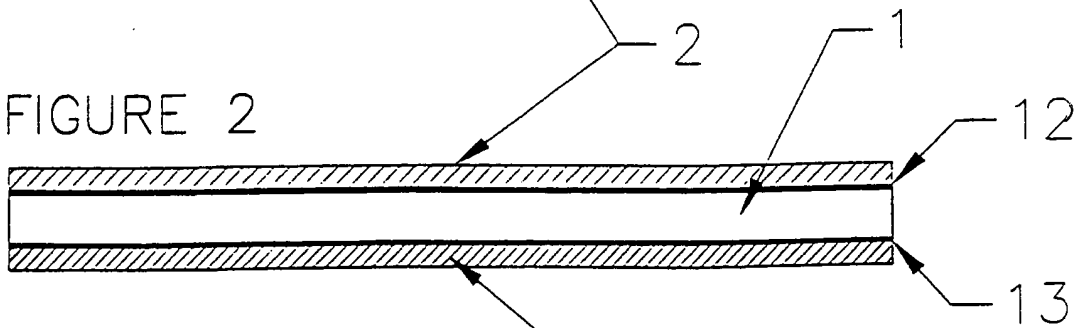
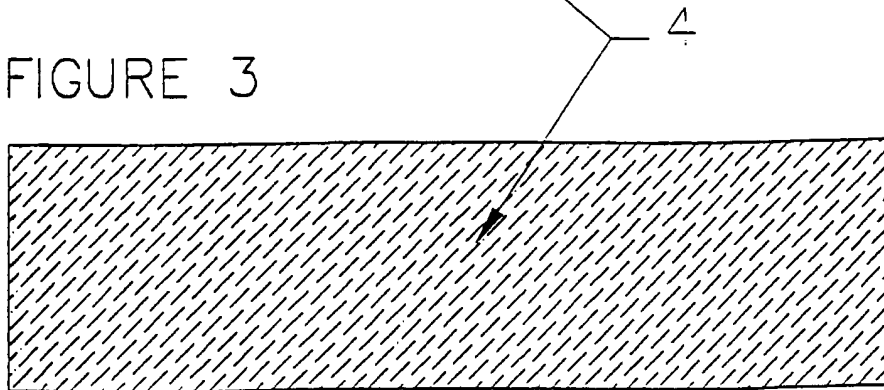


FIGURE 3



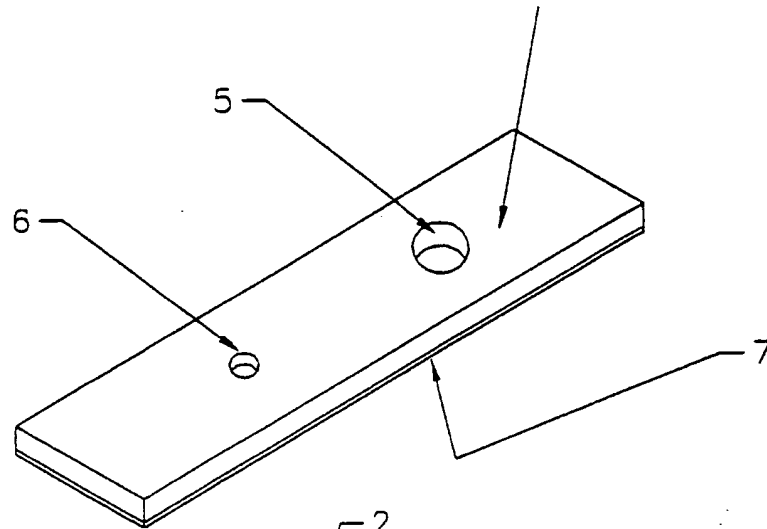


FIGURE 4

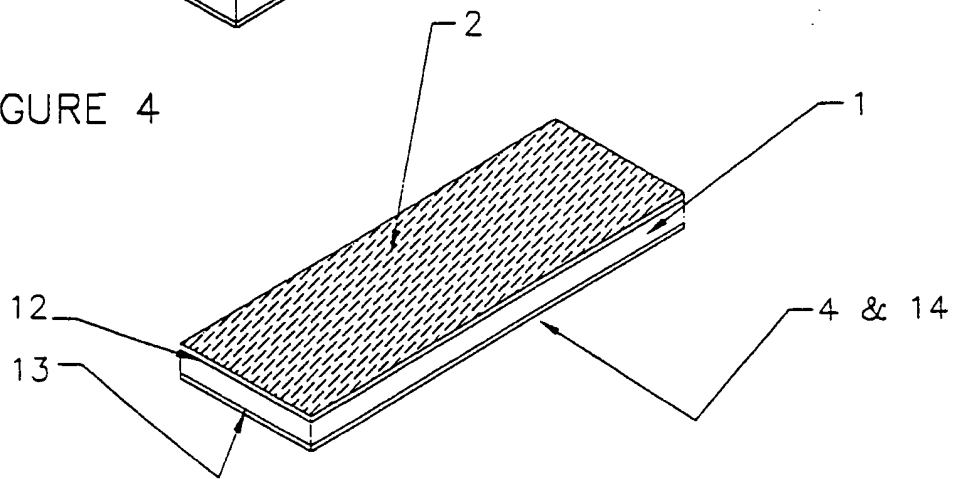


FIGURE 5

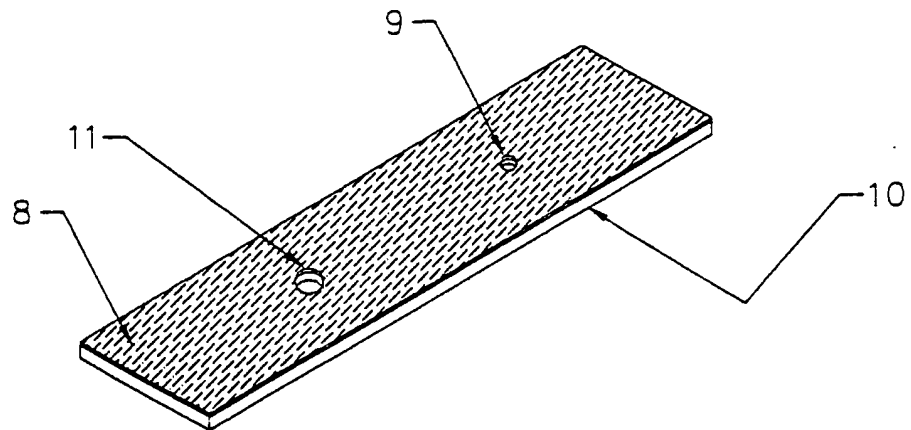


FIGURE 6

3/5

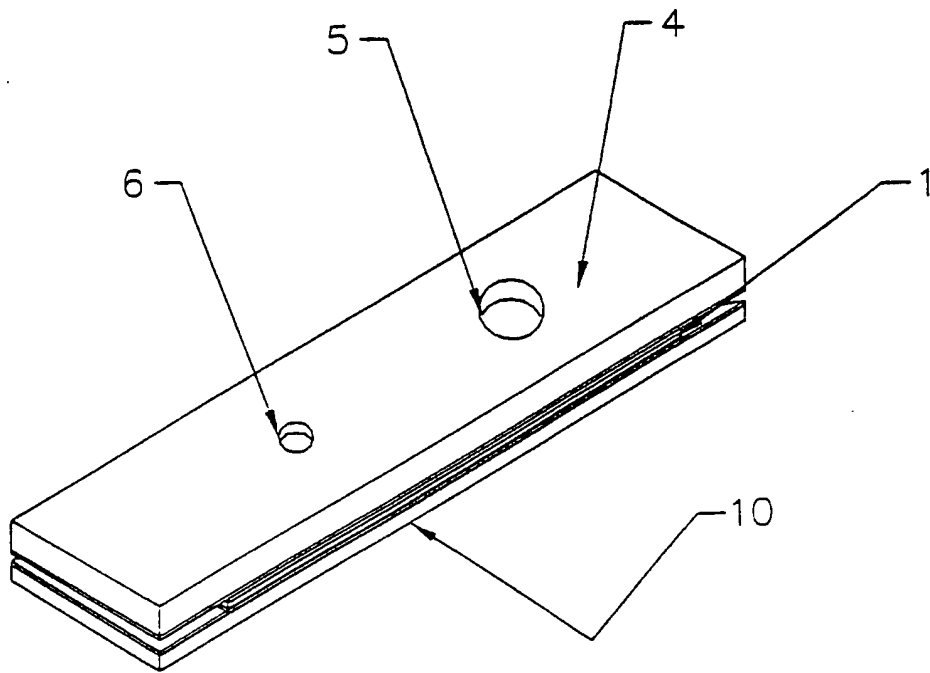


FIGURE 7

4/5

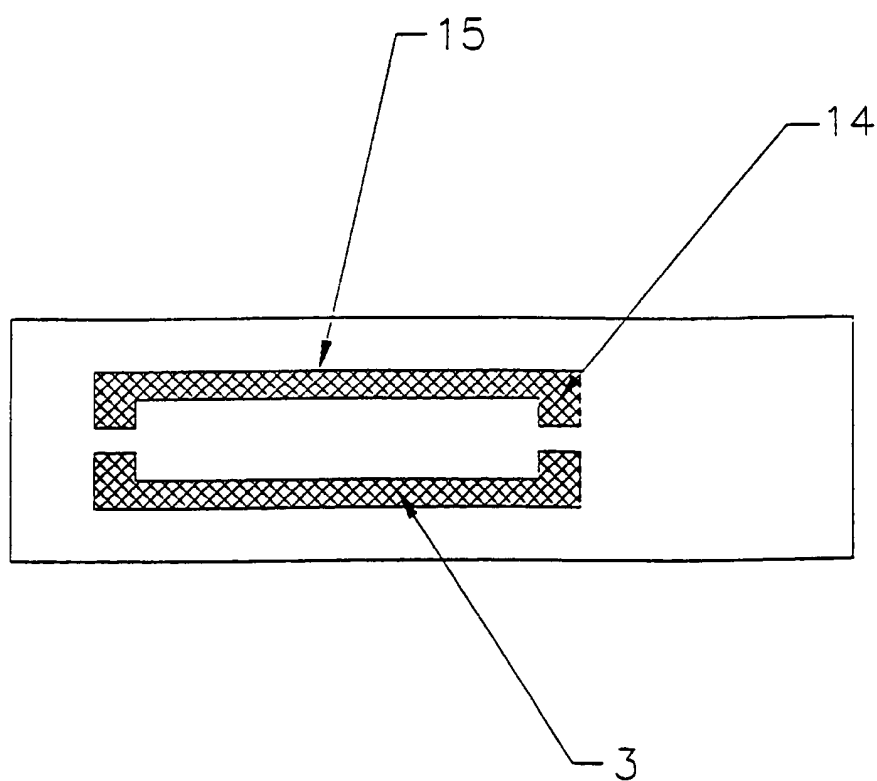


FIGURE 8

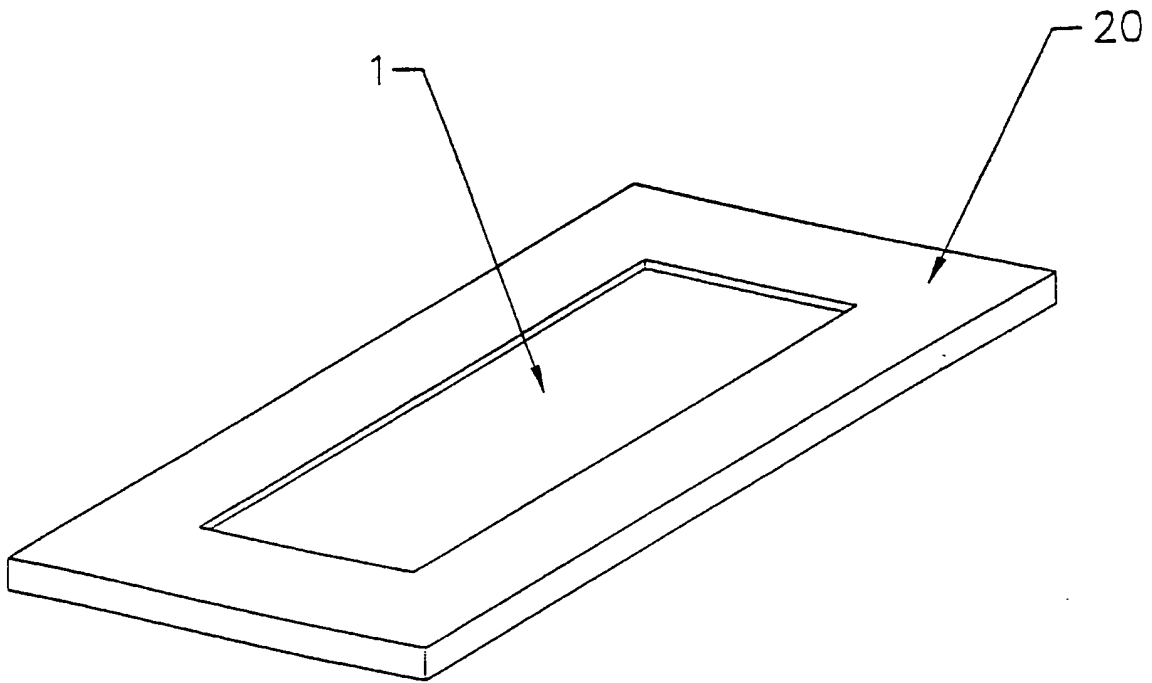


FIGURE 9



# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/20575

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 G01N33/487

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	YOKOYAMA K ET AL: "AMPEROMETRIC GLUCOSE SENSOR USING SILICON OXIDE DEPOSITED GOLD ELECTRODES" ELECTROANALYSIS, vol. 3, no. 4/05, May 1991, pages 469-475, XP002041662 see the whole document ---	1-15
A	PATENT ABSTRACTS OF JAPAN vol. 9, no. 243 (P-392), 30 September 1985 & JP 60 095343 A (HIRATSUKA KAZUYA), 28 May 1985 see abstract ---	1-15
A	US 5 437 999 A (DIEBOLD ET AL.) 1 August 1995 cited in the application ---	1,7,11
-/--		

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

\* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

25 January 1999

Date of mailing of the international search report

01/02/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Bosma, R

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/20575

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DE 43 00 499 A (KLEIN, KARL-DITTMAR, DR.) 14 July 1994 see the whole document ---	1,7,11
A	EP 0 470 290 A (SIEMENS AG) 12 February 1992 ---	1,7,11
A	EP 0 592 805 A (AGENCY OF INDUSTRIAL SCIENCE & TECHNOLOGY, ET AL.) 20 April 1994 see the whole document ---	1,7,11
A	EP 0 351 891 A (MEDISENSE, INC.) 24 January 1990 cited in the application see the whole document ---	1,7,11
A	EP 0 710 835 A (TOYOTA JIDOSHA KK) 8 May 1996 see the whole document -----	1-15

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/20575

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 5437999	A	01-08-1995	CA 2183865 A EP 0753051 A JP 9509740 T WO 9522597 A	24-08-1995 15-01-1997 30-09-1997 24-08-1995
DE 4300499	A	14-07-1994	DE 4330517 A DE 4422018 A	09-03-1995 21-12-1995
EP 470290	A	12-02-1992	DE 59010549 D DK 470290 T JP 4233446 A US 5746898 A	28-11-1996 01-04-1997 21-08-1992 05-05-1998
EP 592805	A	20-04-1994	JP 2090591 C JP 6094671 A JP 8012169 B JP 2071934 C JP 6094670 A JP 7104314 B US 5503728 A US 5704118 A	18-09-1996 08-04-1994 07-02-1996 25-07-1996 08-04-1994 13-11-1995 02-04-1996 06-01-1998
EP 351891	A	24-01-1990	GB 2154003 A AU 572138 B AU 2775584 A CA 1226036 A DE 3485554 A DE 3486221 D DE 3486221 T EP 0127958 A EP 0351892 A JP 9325127 A JP 7072727 B JP 60017344 A US 5682884 A US 5727548 A US 5820551 A AU 616169 B AU 1239188 A US 5509410 A AU 569076 B AU 2775384 A AU 580257 B AU 2775484 A CA 1219040 A CA 1223638 A CA 1218704 A CA 1220818 A EP 0125867 A EP 0125136 A EP 0125137 A EP 0125139 A US 4758323 A US 4711245 A	29-08-1985 05-05-1988 08-11-1984 25-08-1987 16-04-1992 04-11-1993 27-01-1994 12-12-1984 24-01-1990 16-12-1997 02-08-1995 29-01-1985 04-11-1997 17-03-1998 13-10-1998 24-10-1991 07-07-1988 23-04-1996 21-01-1988 08-11-1984 12-01-1989 08-11-1984 10-03-1987 30-06-1987 03-03-1987 21-04-1987 21-11-1984 14-11-1984 14-11-1984 14-11-1984 19-07-1988 08-12-1987
EP 710835	A	08-05-1996	JP 8327590 A EP 0710996 A JP 8329969 A	13-12-1996 08-05-1996 13-12-1996

# INTERNATIONAL SEARCH REPORT

...information on patent family members

International Application No

PCT/US 98/20575

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 710835 A		US 5712052 A	27-01-1998